Modification-specific proteomic analysis of glycoproteins in human body fluids by mass spectrometry - DTU Orbit (08/11/2017)

Modification-specific proteomic analysis of glycoproteins in human body fluids by mass spectrometry: Proteomics of Human Body Fluids: Principles, Methods, and Applications

Glycosylation of proteins is a very common, diverse, and heterogeneous type of modification, especially for proteins with extracellular destinations. This chapter describes some general strategies for the enrichment of glycoproteins and glycopeptides with an emphasis on proteomic analysis of N-glycosylated proteins in body fluids and other complex samples. An approach for identification of N-glycosylated proteins and mapping of their glycosylation sites is described. In this approach, glycoproteins are initially selectively purified by lectin chromatography. Following tryptic digestion, glycopeptides are enriched by hydrophilic interaction chromatography (HILIC). Glycan heterogeneity is then reduced by treating the glycopeptides with endoglycosidases. The resulting peptides are then analyzed by matrix-assisted laser desorption/ionization (MALDI) mass spectrometry and nano-flow reversed-phase liquid chromatography tandem mass spectrometry (LC-MS/MS). The analysis allows the identification of N-glycosylation sites and is demonstrated on a mixture of standard proteins.

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